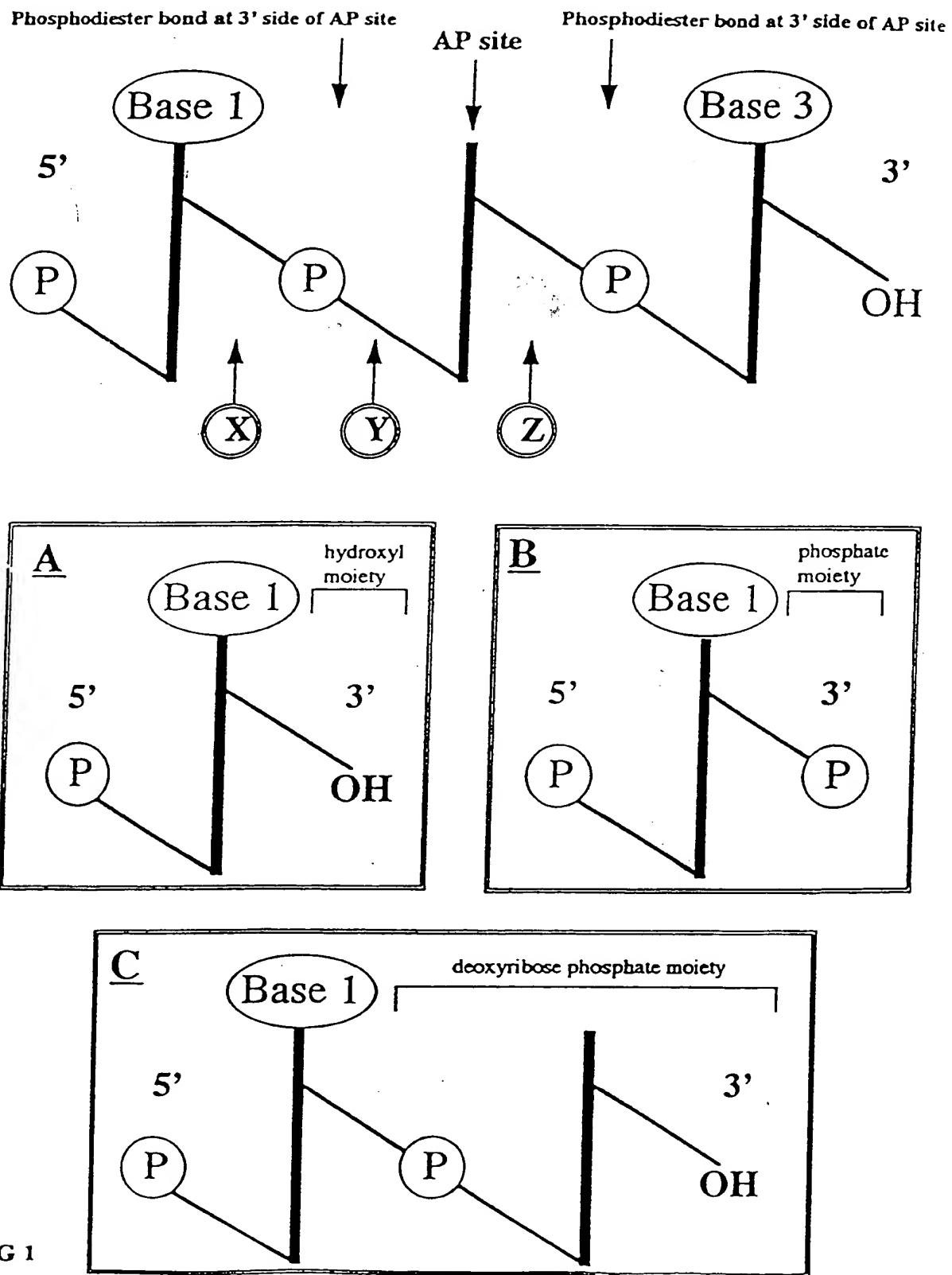
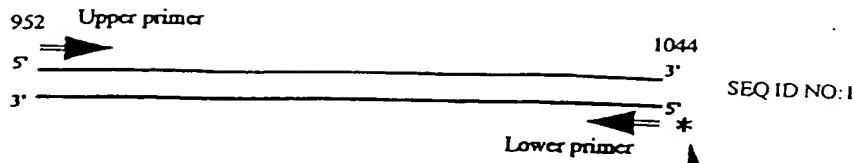


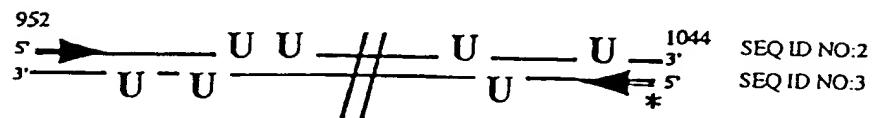
1/7



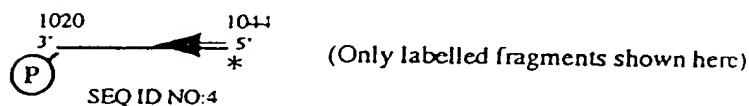
2/7



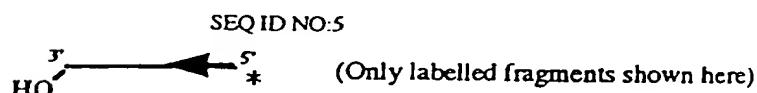
Amplification of target nucleic acid in the presence of dGTP, dATP, dUTP and dCTP and  $^{32}\text{P}$  labelled lower primer.



- Treatment with Exonuclease I and Shrimp Alkaline Phosphatase.  
- Treatment with UDG.  
- Treatment with NaOH at 95°C



Removal of 3' phosphate by treatment with T4 PNK



Linear amplification of DNA (952 to 1044) using labelled upstream fragment followed by analysis on denaturing polyacrylamide gel, followed by autoradiography.

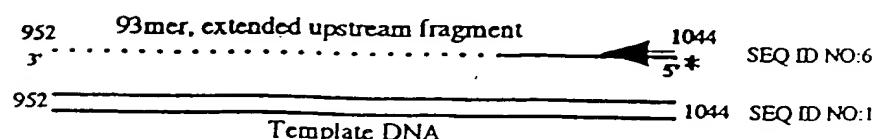
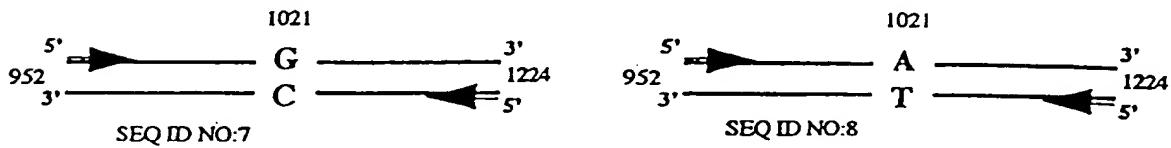


FIG 2

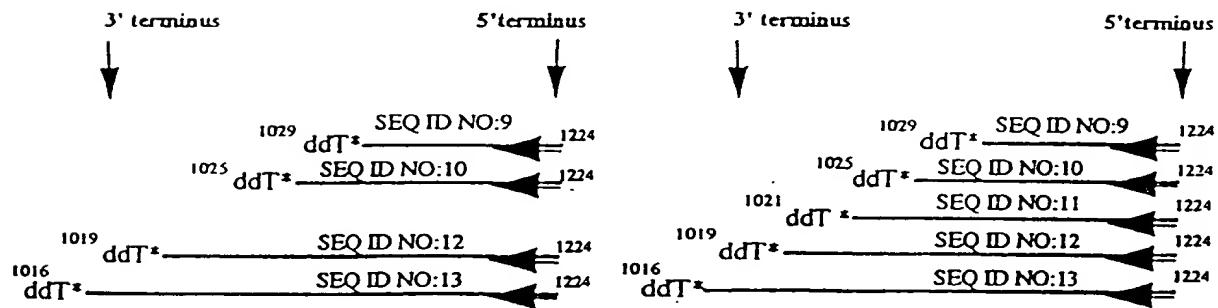
3/7

- Amplification of normal and mutant target nucleic acid in the presence of dGTP, dATP, dCTP and 1/20 ratio of dUTP to dTTP.



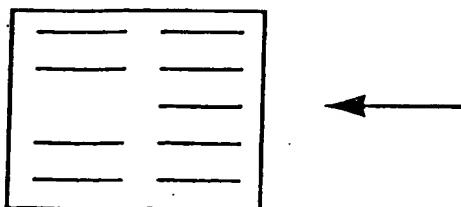
- Treatment with Exonuclease I and Shrimp Alkaline Phosphatase.
- Treatment with UDG.
- Treatment with NaOH at 95°C
- DNA is precipitated.
- Treatment with T4 PNK

Extension of the upstream fragments generated above in the presence of  $^{33}\text{P}$ -labelled ddTTP\* and unlabelled ddGTP, ddATP and ddCTP.



Only some fragments corresponding to cleavage at U incorporation sites surrounding the mutation site are shown here.

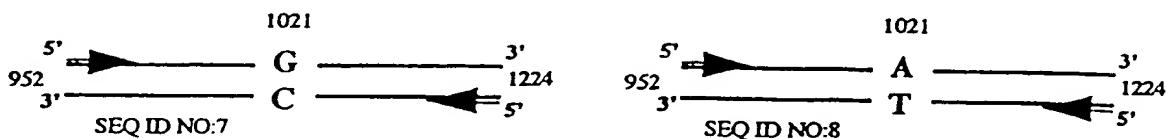
## Detection of extended labelled fragments by PAGE and autoradiography



**FIG 3**

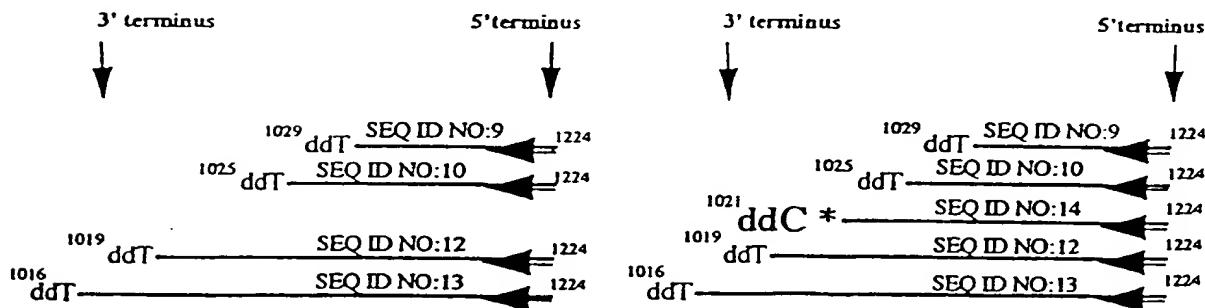
4/7

- Amplification of normal and mutant target nucleic acid in the presence of dGTP, dATP, dCTP and 1/20 ratio of dUTP to dTTP.

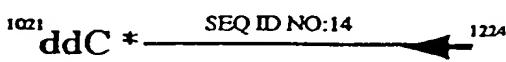


- Treatment with Exonuclease I and Shrimp Alkaline Phosphatase.
- Treatment with UDG.
- Treatment with NaOH at 95°C
- DNA is precipitated.
- Treatment with T4 PNK

Extension of the upstream fragments generated above in the presence of  $^{33}\text{P}$ -labelled ddCTP\* and unlabelled ddGTP, ddATP and ddTTP.



Only some fragments corresponding to cleavage at U incorporation sites surrounding the mutation site are shown here.



Detection of extended labelled fragments by PAGE and autoradiography

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6390      **Upper primer**      **Mutation site**      **SEQ ID NO:15**  
 A      6443  
 5' **AACTTGTGGTAGTTGGAGCTCGTGGCGTAGGCAAGAGTGCCCTGACGATAACAGC** 3'  
 3' **TTAACACCATCAACCTCGACCACCGATCCGTTCTCACGGAACTGCTATGTCG** 5'  
 T      **Lower primer**

Amplification of target nucleic acid in the presence of dGTP, dATP, dUTP and dCTP.

SEQ ID NO:16

### Amplified normal allele

5' AACTTGTGGTAGTTGGAGCTGGUGGCGUAGGCAAGAGUGCCUUGACGAUCAGC 3'  
3' UUGAACACCAUCAACCUCGACCACCGCATCCGTTCTACGGAACTGCTATGTCG 5'  
SEQ ID NO:17

SEO ID NO:18

### Amplified mutant allele

5' AACTTGTGGTAGTTGGAGCTGAUGGCGUAGGCCAAGAGUGCCUUGACGAUACAGC 3'  
3' UUGAACACCAUCAACCUCGACUACCGCATCCGTTCTACGGAACTGCTATGTGCG 5'  
SEQ ID NO:19

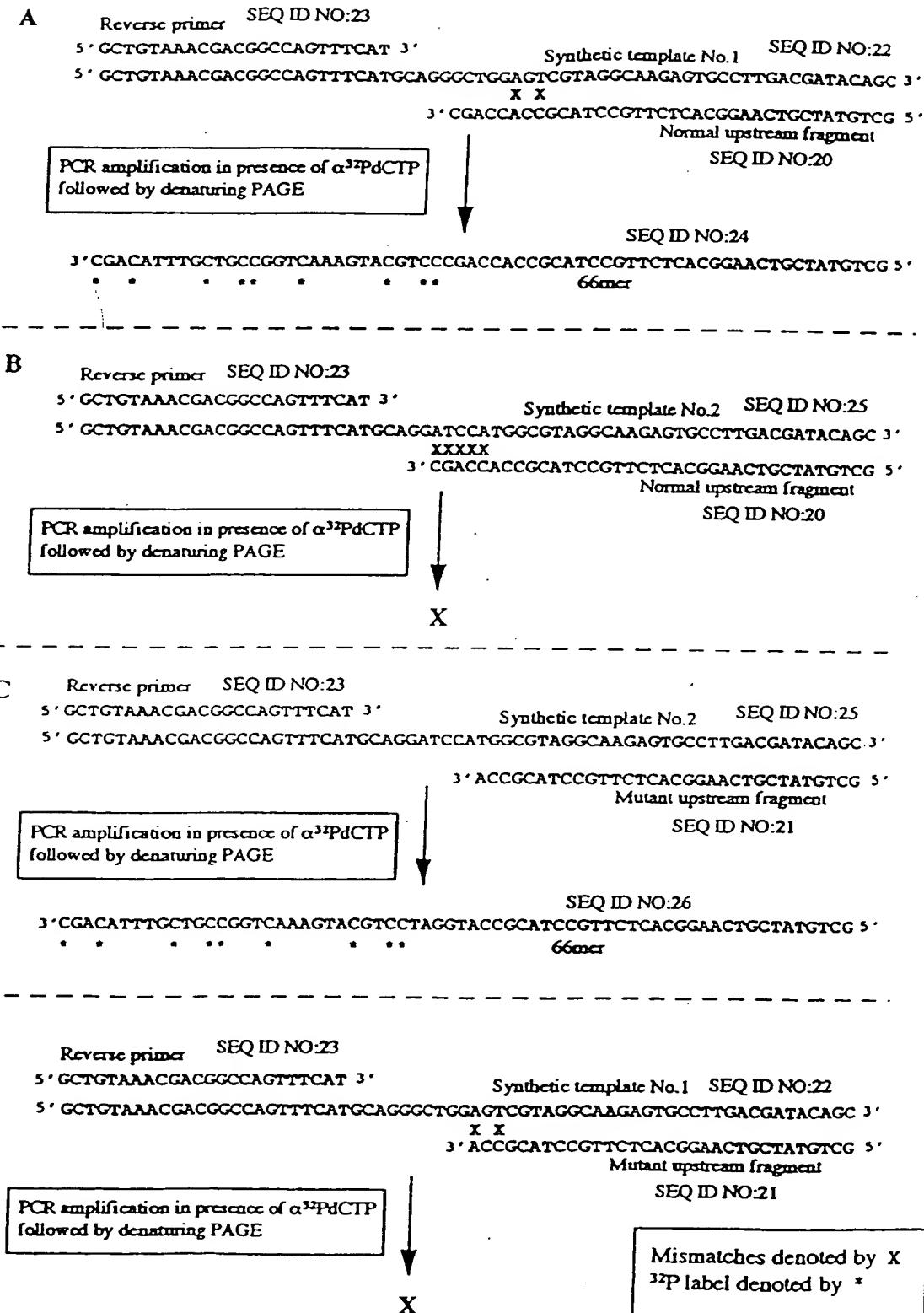
- Treatment with Exonuclease I and Shrimp Alkaline Phosphatase.
- Treatment with UDG.
- Treatment with Endo IV.

SEQ ID NO:20 Normal upstream fragment  
3' CGACCACCGCATCCGTTCTCACGGAAGTGCTATGTGCG 5'

SEQ ID NO:21      Mutant upstream fragment

3' ACCGCATCCGTTCTCACGGAACTGCTATGTGCG 5'

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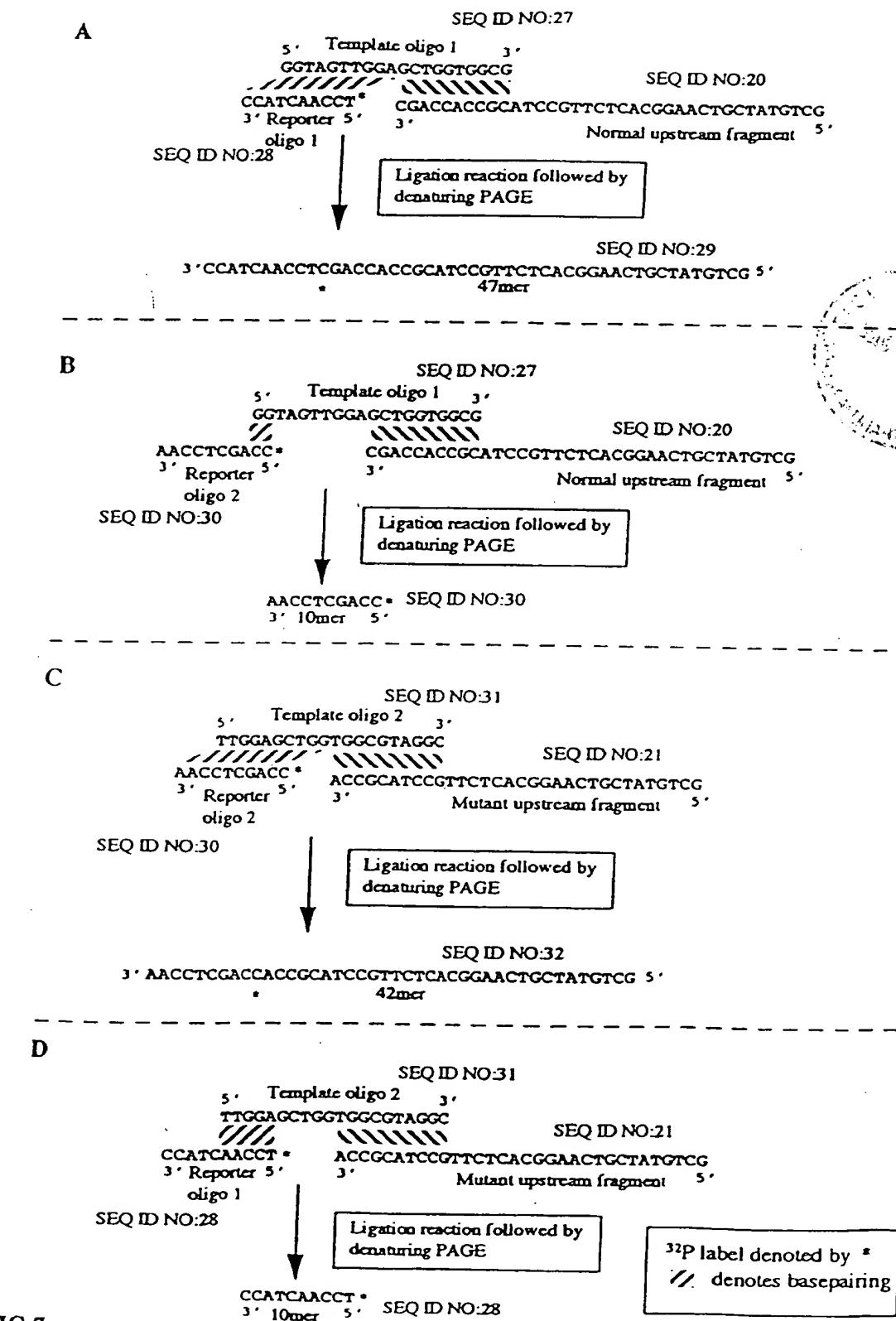


FIG 7